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THE MORPHOLOGY OF ROOT TUBERCLES OF LEGUMINOSÆ.

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The object of this research was to give a more detailed account of the anatomical structure of root tubercles, thus adding perhaps somewhat toward clearing up some of the difficulties in the comparatively little known field of teratology. It is true much labor has been expended in the endeavor to get a correct understanding of "symbiosis" and the relation of *Rhizobia* to certain plants. The life history and morphology of the root bacteria have received most of the attention while the tubercles themselves have been almost entirely forgotten. Frank, who has done much in this line of research offers but little concerning tubercle morphology. Nearly all of his attention is directed toward the "*Bakteroiden.*" The same is true of Beyerinck, Brunchorst, Waronin and the others. Some of the pupils of Frank have also made noteworthy researches concerning Rhizobia. Recently Frank and Moeller[2] have engaged in a controversy with regard to the "*Dimorphismus der Wurzelknöllchen der Erbse.*" Frank has also described the lenticular structure of root tubercles. Beyond this, little of importance seems to have been done with root tubercle morphology.

In my own researches, mature tubercles were taken from various *Leguminosæ* toward the close of the vegetative period, since at that time tissues have acquired their most marked morphological characteristics. The earlier stages of development were studied at various periods of the season. The final summing up was not completed until late in the year. (Dec., 1892.)

The tubercles are in themselves abnormal growths produced by the local irritation of certain organisms, the Rhizobia; and etiologically resemble those growths produced by certain animals as for example the *Heterodora* or *Anguillula* cysts found on some roots. Morphologically, however, they are clearly distinct.

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[1] The investigations described in this paper were carried on in the laboratories of botany, of the University of Minnesota.

[2] Berichte der deutsch. bot. Ges., Hefte 3 und 5, 1892.

In some cases these tubercles make their appearance shortly after germination. In others not until plants have reached the flowering stage or even later. In some cases they do not appear at all, that depending upon the nature of soil in which the plants grow as explained by Frank[3] and others. The macroscopic appearances of some tubercles have been more or less perfectly described in a previous article.[4] The form is quite constant in a given species of plant. In *Phaseolus vulgaris* and *Amphicarpaea comosa*, for instance, they are spherical. In *Melilotus alba, Pisum sativum, Trifolium pratense* and *Trifolium repens* they are oblong, narrow at point of attachment, often slightly forked, cordate or pear shaped. Usually they occur singly, less often, as for example in *Melilotus alba* they grow into large grape-like bunches. Like root branches they usually develop acropetally though there are numerous exceptions. They are most common on roots near the surface of the soil; sometimes they are found at a considerable depth. They are never found in close proximity to growing root tips. They occur either on the main root or its branches.

Tubercles seem always to develop exogenously. The direct causes of their development are the Rhizobia, of which there are at least several species.[5] Predisposing causes are, nature of soil and condition of atmosphere as to temperature and moisture, etc. The various kinds of Rhizobia appear to be more or less abundant in all normal soil. Whenever a given species finds a suitable lodging place on the root-surface it multiplies and by some means gains access to one or more of the surface cells and infection is completed. Just how infection takes place is not definitely known. It is thought that the Rhizobia have the power to dissolve the cell membrane of the host; just how is not stated. Frank, Moeller[6] and others maintain that infection takes place by means of the "*Infektionsfäden.*" This seems improbable since the "*Infektionsfäden*" are not always present. I could find no trace of them in *Phaseolus vulgaris.* They are sometimes present in *Trifolium pratense.* I have been unable to find them in *Robinia pseudacacia,* although Moeller states that they are present. In all those cases in which I found them I could detect no connection between them and the Rhizobia. They are sometimes found in tissues outside of the tubercle having no apparent connection with it. In chemical behavior and microscopical appearance the "*Infektionsfäden*" differ considerably from the Rhizobia. The "*Infektionsfäden*" consist of a more highly refractive albuminoid substance and stain less readily than the Rhizobia. I have often found them in old tubercles of *Trifolium pratense* in which the Rhizobia and cell protoplasm had been almost wholly removed showing that they have a greater vitality than either the plant cell or the Rhizobia. Heretofore no one has been able to detect a membrane on the "*Infektionsfäden.*" Recently Moeller[7] has announced the discovery of a cellulose membrane surrounding the "Bacteria zoogleæ"—"*Infektionsfäden.*" This membrane, he asserts, is secreted by the cell protoplasm as a protection against the intruding bacteria (Rhizobia). In the same article Moeller notes that the bacteria (Rhizobia) have the power to dissolve cellulose. These statements are certainly a little difficult to understand. It would seem improbable that the bacteria (Rhizobia) should at one and the same time dissolve cellulose and also have a coating of it deposited on them as a protection against their intrusion. It is known that eggs of parasites, larvæ in the resting stage, calcium oxalate crystals, etc., when found in plant tissues have had secreted around them a coating of cellulose. In such cases, however, the foreign substance does not at the same time have the power of dissolving cellulose.

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[3] Pilz symbiosa der Leguminosen, 1890.

[4] Bulletin Torrey Bot. Club, July, 1892.

[5] Bulletin Torrey Bot. Club, July, 1892.

[6] Ber. der deutsch. bot. Ges., Heft 5, 1892.

Without going into further discussion, it is known that the Rhizobia gain access to the interior of the surface root-cells where they multiply rapidly. By their irritating presence, increased protoplasmic action takes place. Not only do the

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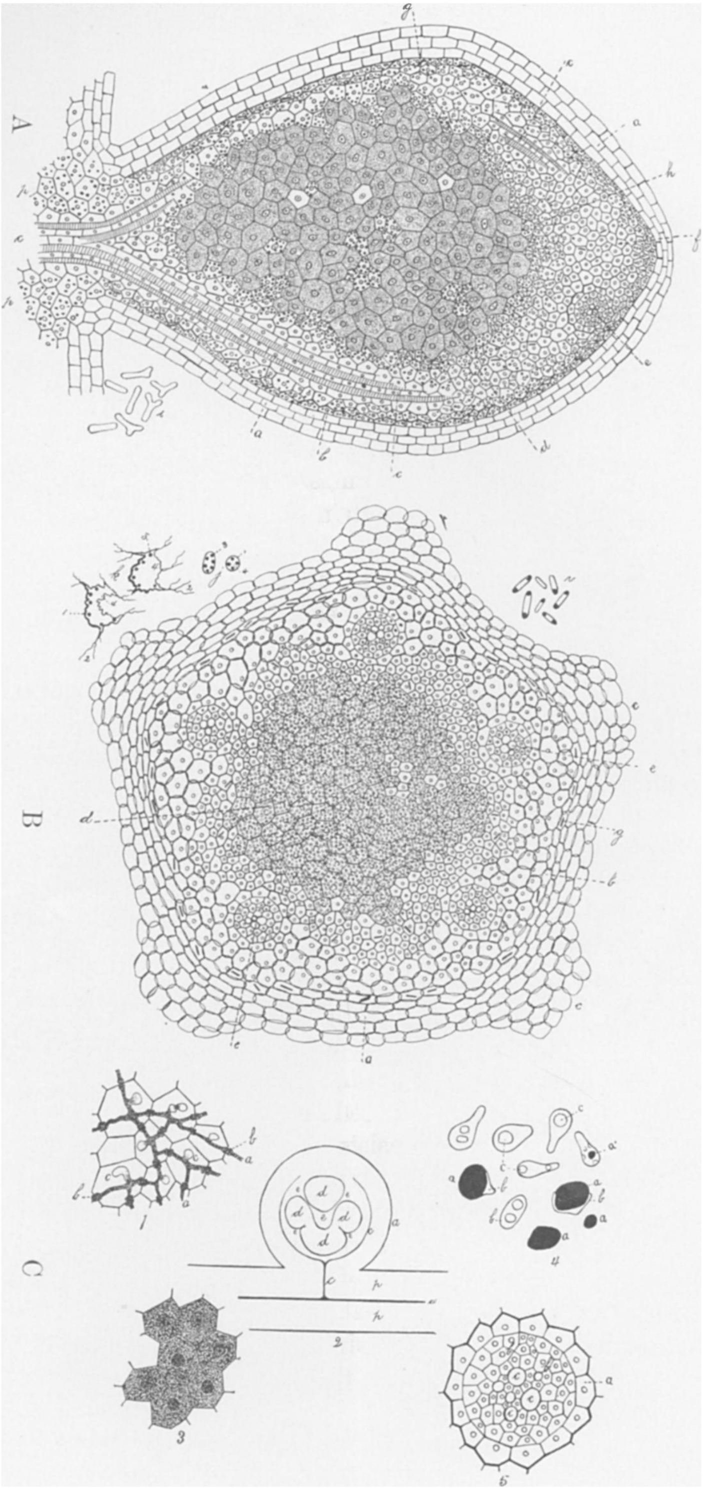
[7] Ber. der deutsch. bot. Ges., Heft 5, 1892.

PLATE XVII.



Root Tubercles of Leguminosæ.

infected cells grow and multiply much more rapidly but the adjoining cells likewise take on increased activity. On microscopic examination there will be noted a slight protuberance, swelling outward but still covered by the root epidermal layer. This incipient tumor consists of two kinds of meristematic tissue, the centrally located portion of infected cells and the adjoining outer portion of noninfected cells. This meristem tissue is engaged in active cell division. The original infected cells divide and produce auto-infected daughter cells. Outwardly a corky tissue is developed from a distinct phellogen layer, usually one or two cells in thickness. These phellogen cells divide tangentially and often contain starch and calcium oxalate crystals. I have found a cork layer on all tubercles examined thus far. Centrally cell division goes on less rapidly until the pericambium of the root is reached which is also involved in the change. A vascular bundle starts from the root-vascular system and extends peripherally toward the infected area until quite near it where it divides into from four to seven branches which continue centrifugally (in reference to root) through the outer part of the tubercle meristen to near its apex. These vascular bundles consists of conducting vessels, like those of the root, surrounded by bast cells: the whole is encircled by a sheath of cells whose outer walls are considerably thickened.

In spherical tubercles the cambium is of equal thickness in all parts. In elongated and irregular forms the meristematic tissue is more abundant in the extended points, that is, there may be one or more apical areas in the same tubercle. The cambium cells are of the ordinary small, angular, closely united, rather thin walled variety. The cells may divide in any plane. The cork layer usually consists of thin walled rectangular cells, often with intercellular air passages. In *Phaseolus vulgaris* the cork cells are considerably rounded, in Robinia they are rather irregular. The entire cork layer is continuous with the unchanged endodermal layer of the root. The entire tubercle is covered over by an epidermal layer of cells contiguous with that of the root. No root-hairs are present. Early in the development of the tubercle the root-hairs become dwarfed and soon disappear. The plant can no longer use them as organs of absorption on account of the impervious layer of cork. The tubercle receives its nourishment by means of the vascular bundle system and the parenchymatous conducting tissue of the root which is continuous with that of the tubercle. The outline of the infected area is wavy, there being a depression before each vascular bundle as seen in a cross section. Single, or groups of noninfected cells are generally distributed through the infected area. In the centre may be found a noninfected area of considerable size, as in *Robinia pseudacacia.* The infected cells resemble those of the noninfected cambium in that they are angular and closely united, there being no intercellular spaces. They differ in that they are much larger and in that they have undergone peculiar protoplasmic and nuclear changes. The cells are entirely filled with Rhizobia and protoplasm. The Rhizobia feed upon the cell protoplasm and in turn appropriate for the use of the plant the free nitrogen of the air. In the case of *Rhizobium mutabile* the abundant food supply causes it to become much enlarged and to assume various forms during the season, hence the name. No other species seem to undergo such extensive changes. As to the position the Rhizobia take in the cell, I have noticed some peculiarities. In colonies of *Rhizobium mutabile* in *Melilotus alba* the long axes are all directed toward the nucleus. *Trifolium* which contains the same species of Rhizobium does not show the same arrangement. Here they are placed in all concievable positions. Why they should take this position in *Melilotus alba* I am unable to state. In *Phaseolus vulgaris* and *Pisum sativum* they are often collected into Zoogloeae as is clearly shown in thin sections.

In *Amphicarpaea comosa* and *Phaseolus vulgaris* I have always found well developed lenticels on the tubercles. They are developed from a clearly marked lenticular phellogen, showing tangential cell division. The phellogen of the lenticels as well as that of the cork layer is developed from a large celled parenchymatous tissue lying above the cambium layer. The lenticular phellogen is not depressed into the underlying tissue, as is usually the case, but somewhat elevated above it. The lenticels consist of the usual loosely connected, rounded cork cells and are always located above the vascular bundles. They no doubt serve as a means for the interchanging of gases between the exterior and interior by way of the vascular bundles. Frank maintains that the cork layer as well as the infected area have intercellular air spaces for the interchange of gases. As already mentioned the infected area consists of closely united firm walled cells, such as are usually found in meristematic tissues. Furthermore the infected area is entirely cut off from the cork layer by means of the cambium which certainly has no intercellular passages. Hence it would be rather difficult to see what function intercellular spaces would play in the infected area. Tubercles possessing lenticels no doubt give off considerable gas. I have noticed that the tubercles of a growing bean plant when placed in water would have a glistening appearance due to a thin layer of air or some gas separating them from the water. After a time very minute gas bubbles would form on the surface of the tubercle. As to the source and nature of this gas I am unable to give any satisfactory explanation. It is very likely dependent upon the largely increased metabolic processes going on in the tubercle, Frank has made some experiments on the subject without however, coming to any definite conclusions.

Starch is usually present to some extent in tubercles, especially in those infected by *Rhizobium mutabile.* There is some in tubercles of *Amphicarpaea comosa,* little or none in tubercles of *Phaseolus vulgaris* and *Robinia pseudacacia.* The starch is always found in the noninfected meristem tissue especially the cork phellogen and next to the infected area. This is simply *stored starch* like that found in other parts of the root.

Frank maintains that there are two varieties of Rhizobia always to be found in two different kinds of tubercles of *Pisum sativum.* One variety is said to have the power of producing within itself highly refractive amyloid bodies closely related to amylo-dextrin found in some of the Florideae. Moeller agrees with Frank in regard to the presence in some Rhizobia of the highly refractive bodies but maintains that they consist of some fatty derivative, as cholesterin. Both agree that these bodies are readily stained with iodine solution, but while Frank notes a reddish brown stain Moeller affirms the color to be dark brown. I have noted similar highly refractive bodies in *Rhizobium mutabile* of *Melilotus alba, Trifolium pratense, Trifolium repens,* and *Lathyrus odoratus* but not in *Phaseolus vulgaris,* or *Pisum sativum.* These two last named plants are infected by *Rhizobium Frankii* var. *major* and *minor* respectively. So far I have only found these bodies in *Rhizobium mutabile.* These bodies are, as before stated, highly refractive; generally rounded, and may be located in the centre or toward the periphery of the usually more or less modified *Rhizobium.* I have been unable to stain them with tincture of iodine. Fuschin stains them with difficulty. The various aniline dyes have practically no effect upon them. As to their chemical nature I am not prepared to express a definite opinion. I am of the opinion that they are not starch. I am more inclined to Moeller's view that they are due to a fatty degeneration of protoplasm in improperly nourished Rhizobia. This would especially be expected in mature degenerating tubercles, which are the only ones that contain the Rhizobia with refractive bodies. Fatty degeneration is quite common in animal protoplasm. It sometimes happens that a starch granule is deposited inside of the partially or wholly empty case of a Rhizobium. This however is purely accidental and does not occur often. Of course such Rhizobia will contain highly refractive bodies that stain readily with iodine.

The greatest changes take place in the infected area of the tubercle. Here everything points to increased protoplasmic activity. The cells grow and multiply rapidly, they are entirely filled with a mixture of Rhizobia and protoplasm and in some cases the "*Infektionsfäden.*" The nuclei are abnormally large, the nucleoli become more distinct. All the different cell elements stain much more heavily than those of the normal cell. In *Phaseolus vulgaris* the nuclei becomes very much modified, they increase in size, the nuclear wall thickens, sooner or later the weaker spots of the wall give way and allow the nucleoplasm to protrude giving the whole an amoeba like appearance. Finally the nuclear wall ruptures on one side and allows the nucleoplasm to escape and mix more or less with the cell protoplasm (cytoplasm). The nucleoli retain their normal size and form; some of them retain a position next to the nuclear wall, others escape into the "mycoplasm" of Frank. I have not noticed such extensive nuclear changes in any tubercles except those of *Phaseolus vulgaris.*

The question as to what becomes of the contents of the infected cells might be briefly considered. Formerly I expressed it as an opinion that the plant at the close of the vegetative period and also at other suitable times absorbed the protoplasmic contents of the Rhizobia. I do not now think that that is the rule if it occurs at all. I believe that normally the tubercles are destroyed by the ordinary process of decay and the Rhizobia thus liberated. I have found the usual number of tubercles on *Trifolium pratense* and *Melilotus alba* as late as December 10th when I was compelled to chop them out of the frozen ground. The tubercles were filled with *Rhizobium mutabile,* apparently in normal condition. I have found well filled tubercles on dead, matured plants of *Phaseolus vulgaris.* The Rhizobia were apparently in a resting stage, waiting to be liberated. It is true in some cases the tubercles were emptied during or before the close of the vegetative period. Even in such cases I do not believe that the contents were taken up by the host but rather that the tubercles died and decayed because it did not receive enough nourishment from the plant and soil.

The question as to what position the tubercles hold, morphologically considered, is of some interest. The absence of a root-cap, of root-hairs and the fact that it develops exogenously would show that it does not resemble a root-branch. In position it shows neither positive nor negative geotropism though it occurs most frequently on roots near the surface. That may be, as Frank maintains, because the tubercles require a coating of air to keep them from direct contact with moisture. But since the tubercle is nourished by the plant and the coating of air escapes from the tubercle itself it is difficult to see why they should not develop in deep soil as well. It may be that there is a tendency toward negative geotropism. I have never been able to find tubercles on any other part of the plant than the root or its branches. Frank has found Rhizobia in stem, leaves and even the embryo. I have searched for them repeatedly in tissues outside of the root but have not found any. Tubercles resemble growing tissues in the abundance of Albuminoid compounds. Calcium oxalate crystals are common in the noninfected cambium especially the cork phellogan. Some of the crystals have a coating of cellulose secreted around them by the cell protoplasm. Any abnormal growth resembles histologically the tissue upon which it develops; hence one would expect the tubercles to resemble anatomically the roots upon which they grow. Yet it seems that the tubercles have more anatomical characters belonging to a stem than to a root, as already indicated.

RESUME.

1. Root tubercles develop exogenously.
2. Tubercles grow from a meristematic area surrounding the infected region and separating it from the external cork layer.
3. Cork as well as lenticles develop from a well marked phellogen.
4. Tubercles have a well developed vascular system differing from that of the root.
5. *Phaseolus vulgaris* and *Amphicarpaea comosa* have tubercles with well developed lenticels, differing somewhat from those on the stem.
6. There is more or less starch in tubercles produced by *Rhizobium mutabile.*
7. Sometimes *Rhizobium mutabile* contains highly refractive bodies whose nature is not definitely known.
8. Anatomically a tubercle resembles a stem more closely than a root.
9. Generally tubercles are not found empty at the close of vegetative period.
10. Probably the plant does not reabsorb the protoplasmic contents of the Rhizobia at any time.
11. Nuclei in tubercles of *Phaseolus vulgaris* undergo great changes.

EXPLANATION OF PLATES.

A. Longitudinal section of a mature tubercle of *Melilotus alba.*

- a. Cork layer.
- b. Phellogen.
- c. Vascular bundle in longitudinal section.
- d. Infected area showing the Rhizobia radiating from the nucleus as a centre.
- e. Vascular bundle, cross section.
- f. Apex of cambium layer.
- h. Starch layer next to infected area.
- i. *Rhizobium mutabile* of *Melilotus alba* as seen in the early part of November.
- j. Calcium oxalate crystals.
- k. Parenchymatous tissue continuous with that of the root.
- r. Noninfected cells in the infected area bearing starch.

B. Cross section of a tubercle of *Phaseolus vulgaris.*

- a. Cork layer consisting of rounded cells.
- b. Phellogen.
- c. Lenticels.
- d. Infected area, Rhizobia are arranged in zoogloeae.
- e. Vascular bundles, all in cross section.
- g. Calcium oxalate crystals.
- i. *Rhizobium frankii* var. *majus* from bean tubercle.
- j. Normal nucleoli. 1. Nuclear wall. 3. Nucleoli. 4. Nucleoplasm.
- k. Much modified nuclei form infected area. 1. Nuclear membrane. 2. Processes of the nucleoplasm. 3. Nucleoli. 4. Nucleoplasm.

C. Details.

1. Small portion of tubercle of *Trifolium pratense* invaded by the "Infektions fäden" (*Schinzia leguminosorum*). a. Hyphæ of the fungus. b. Enlargements next to cell wall. c. "Haustoria."

2. Diagrammatic longitudinal section of *Robinia pseudacacia.* a. Cork layer. The cells are more irregular than those of the root. c. Vascular system. d. Infected area. e. noninfected areas.

3. Same as 1, but without the " Infektions fäden " and showing the cell entirely filled with the much modified *Rhizobium mutabile.* Nuclei are much enlarged and nucleoli show distinctly.

4. b. Much modified *Rhizobia mutabilia* with highly refractive bodies c. a. Starch granule which is capped by the empty case of a Rhizobium, b. á. A starch granule just forming inside of a partially empty Rhizobium case. a. Some free starch granules.

5. Cross section of a vascular bundle. a. Sheath. b. Phloem. c. Vessels.